PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Christiane HOLLMAN *et al.*

Serial No.: 10/599,512

Filed: September 29, 2006

For: MONOCLONAL ANTIBODIES WITH SPECIFICITY FOR FETAL ERYTHROID CELLS

Confirmation No.: 1118

Group Art Unit: 1641

Examiner: Gailene Gabel

Atty. Dkt. No.: ONCO:006US

CERTIFICATE OF ELECTRONIC TRANSMISSION

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

06/30/10
Date

Steven L. Highlander

DECLARATION OF WINFRIED ALBERT UNDER 37 C.F.R. §1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, the undersigned, do declare that:

1. I am the former managing director for the assignee of the above-captioned application, Adnagen AG, as well as a named inventor thereon.

2. I have reviewed the Tuma *et al.* reference cited by the examiner in the February 1, 2010 office action.

3. I have personal knowledge of the subject matter described in the Tuma *et al.* patent application since AdnaGen AG has acquired their patent rights and with it the respective knowledge.

4. The antibodies described by Tuma *et al.* as reacting against fetal cells with specificity against the i-epitope lacto-*N*-hexaosylceramide were derived from an auto-antibody of a leukemia patient whose serum reacted not only with the patient's leukemia cells, but also with fetal cells. Tuma *et al.* did not produce monoclonal antibody or polyclonal serum produced against i-epitope lacto-*N*-hexaosylceramide, either at the time the application was filed, nor, to my knowledge, thereafter.

5. In contrast, the antibodies being presently claimed do not react with the i-epitope lacto-*N*-hexaosylceramide. Therefore, it is most likely that the specificity of the presently claimed antibodies is distinct from the auto-antibody described by Tuma *et al.*. The leukemia cell line K-562, tested for reactivity, failed to be recognized by the presently claimed antibodies.

6. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that wilful false statements are punishable by fine or imprisonment, or both, under § 1001 of title 18 of the United States Code.

St. Gilgen, June 25, 2001

Date

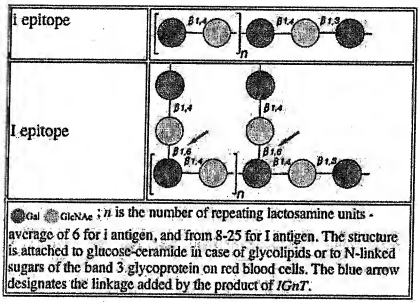
Winfried Albert, Ph.D.

I (I) Blood Group System

Gene locus - GCNT2 (IGnT)
Alleles

Introduction

The I antigen, together with the i antigen, used to be comprised in the Ii blood group collection. However, the gene encoding the I beta-1,6-N-acetyl glucosamine transferase (I beta-1,6-GlcNAcT, GCNT2) responsible for converting i active straight chains of carbohydrates to I-active branched chains has been cloned (Bierhuizen *et al.*, Genes Dev. 7: 468,1993) and, some mutations responsible for adult i phenotype, identified (Yu *et al.* Blood 98:3840, 2001; Yu *et al.*, Blood 101:2081, 2003; Inaba *et al.*, Blood, 101:2870, 2003). Hence, I has been promoted to the system status, the I blood group system, which comprises only a single antigen, the I antigen.



The i and I antigens are carbohydrate structures characterized, as linear and branched repeats of N-acetyl lactosamine, Galbeta1-4GlcNAc1-3Galbeta1-4GlcNAc-R and Galbeta1-4GlcNAc1-3 (Galbeta1-4GlcNAcbeta1-6))Galbeta1-4GlcNAc-R, respectively (poly LacNAcs). These glycans reside on O- or N-linked glycans of extracellular domains of erythrocyte membrane proteins and on membrane glycosphingolipids. In analogy to the ABO, H/h or Lewis systems, the gene loci encode glycosyltransferases responsible for the synthesis of the actual epitopes. The i antigen, the precursor of I, is synthesized by the sequential action of two glycosyl transferases, beta-1,3-acetyl glucosaminyl transferase and beta-1,4 galactosyl transferase. The I antigen is a branched form of the linear i antigen, branching being initiated by a third transferase, the I beta 1,6 GlnAc T, the I branching enzyme, also known as GCNT2; this enzyme differs from other branching beta 1,6 glycosyl transferases (Yeh *et al.*). The expression of the I and i antigens reflects a reciprocal relationship that is developmentally regulated. Adult human erythrocytes (RBCs) fully express I antigens and contain only a few i antigens; the latter predominate in fetal and neonatal RBCs. After birth, the quantity of I antigens gradually increases as the level of i antigen falls, until the normal adult Ii status is reached, about 18 months of life. Most adult RBCs fully express the I antigen; however, in a small number of individuals, only very low levels of I antigen can be detected and their RBCs show high levels of the i antigen. This phenotype is called the "adult i" and is believed to result from lack of activity of the I branching transferase, product of IGNT locus.

In analogy to the other carbohydrate blood group systems, in addition to erythrocyte membrane proteins and glycolipids, the i and I epitopes reside on water soluble glycoproteins of secretions, including saliva, milk, plasma, gastric juice, ovarian cyst fluid and amniotic fluid. In these tissues the expression of I,i is also developmentally regulated but is independent of the I phenotype of the erythrocytes. Thus, normal quantities of the I antigen have been observed in milk, saliva and plasma of individuals with the adult i phenotype, suggesting that different I-branching enzymes may be responsible for i-antigen synthesis in different tissues.

The genes

The locus responsible for the formation of the blood group I antigen is the IGNT gene (Bierhuizen *et al.*) Studies of DNA from individuals with the adult i phenotype (Yu *et al.*) suggested that the locus expresses, through the use of alternative promoters, three IGNT forms IGnTA, IGnTB and IGnTC (1209, 1203, 1209bp respectively) and each encodes a protein of 402 or 400 residues; the three proteins share identical carboxy terminal regions, encoded by identical exons 2 and 3, but differ within the amino terminal regions, encoded by exons 1 (~66% sequence identity). The three isoforms are differentially expressed in various human tissues. Recently, another group of investigators reported similar findings (Inaba *et al.*). They propose that a single IGNT gene gives rise by alternative splicing to three isoforms designated IGNT1, IGNT2 and IGNT3. Those three isofoms are equivalent to IGNTA, IGNTB and IGNTC. Whether a single gene or three distinct genes result in these transcripts and whether they are generated by alternative splicing is still not fully clear and needs further study. Recently, adaptive evolution was proposed to play a role in creating multiple variable first exons resulting in IGNT diversity (Li and Wu, PMID: 17475008). The molecular basis proposed for the expression of IGNT locus offers a new perspective for the formation and expression of I antigen in different cells, and supports the proposition for the existence of more than one I-branching enzyme (Yu *et al.*). Studies of IGNT in reticulocytes and lens-epithelium cells of two groups of adult i individuals, with and without congenital cataracts, showed that the IGNTC (IGNT3) form is responsible for the expression of the I antigen on RBCs and provided a molecular basis for the partial association of the adult i phenotype with congenital cataracts (Yu *et al.*).More recntly it was shown that the activity of IGnTC resulting in branching, during erythroid differentiation, are regulated by the transcription factor CCAAT enhancer binding protein alpha(C/EBPalpha) (Twu et al. Blood2007, 110,4526-4534).

Function of proteins

Initiation of branching of poly-LacNAc glycan units on O- and/or N-linked saccharides of membrane proteins and lipids. Receptors, ligands in adhesion processes.

Tissue distribution

Erythroid cells, lymphocytes, monocytes, granulocytes, platelets, secretions, lens epithelium and other tissues. Differential expression of specific transcripts in different tissues. For example, of IGNTC in erythroid tissues and of IGNTB in lens epithelium.

Disease association

Anti-I is associated with cold agglutinin hemagglutinin disease; decreased expression of I and increased expression of i antigens is observed in oncogenesis, thalassemias, sickle cell anemias and is associated with congenital cataracts in Asian populations.

About the alleles

The existence of a human I genetic polymorphism was first indicated by the discovery of adult

individuals whose erythrocytes were I negative but i positive and who carried a cold-agglutinating anti-I antibody. The I negative phenotype, known as "adult i," is rare in world populations; it has been reported to be associated with congenital cataracts in Asians. Its molecular basis was recently elucidated in two groups of individuals; six adult i caucasian individuals with no cataracts and five adult i Taiwanese individuals with congenital cataracts (Yu *et al.*); also, in three adult i individuals with congenital cataracts and five individuals with common I phenotype (Inaba *et al.*). As noted above the processing of IGnT results in three transcripts IGnTA, IGnTB, IGnTC that differ at the amino terminus but are identical in the carboxyl terminal regions.
In the list of alleles, GenBank sequence with acc no. NM_00149 is used as reference (coding region starts at nt. 709);however, as noted above, gene transcripts occur as isoforms that share exons 2 and 3 but differ in exon 1; because the numbers of nt in the 3 isoforms differ (the number of nucleotides in exon 1 of IGnTB is decreased by 6 (2 fewer amino acids) reference sequences for the isoforms are included and are used when the respective forms of alleles are known or AF458024 for IGnT A, AF458025 for IGnT B and AF458026 for IGnT C. When in an allele, DNA variation occurs in exon 1 it may or may not be found in all 3 isoforms; however if it occurs in exons 2 or 3, it will occur in all 3 isoforms but at correspondingly different positions.Thus, to obtain the position of the same nucleotide change, for IGntB substract 6bp from the position shown for IGntA and/or C; if shown in IGnTB, add 6nt for the corresponding position in A or C. In the Table of alleles, nucleotide changes are shown as indicated in the original publications.

Other database IDs and links

NCBI genes
2651
Uniprot ID
Q06430
Genbank proteins
NP_663624 isoform A
NP_001482 isoform B
NP_663630 isoform C
Gene nomenclature database ID
4204
Genbank nucleic acids
NM_001491 for isoform B
NM_145649 for isoform A
NM_145655 for isoform C
NCBI homologenes for homologs and orthologs
77359
NCBI dbSNP for single nucleotide polymorphisms
2651
OMIM ID - at Online Mendelian Inheritance in Man
600429
110800 for I system

Literature

New PubMed entries with the terms *I (I) and blood* from the last 30 days.
NCBI Book Sections with the terms *I (I) and blood.*

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Contributors for specific alleles are listed with the alleles.

Links

Alleles
More blood group systems

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